CHEMICAL AND PHYSICAL CHANGES DURING GEOTROPIC RESPONSE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 262

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Introduction

The work reported in this paper was undertaken with the object of making as complete a study as possible of all the chemical and physical processes that might be involved in geotropic response. It was hoped in this way not only to add something to the knowledge of the mechanics of geotropic bending, but also to find some quantitative differences which are associated with the differing rates of growth of the two flanks of the responding organ. It became necessary to drop the work before it was complete. Such results as were obtained are reported in the hope that they may prove of some value to others interested in the problem.

Several studies of one or more of the factors which might be involved have been made. KRAUS (8) found that the water content of the convex flank of organs stimulated geotropically is greater even before bending begins. He also made determinations of reducing sugars and titration acidity on the juice expressed from the organs. He concluded that when a stem capable of negative geotropic response is laid horizontally, increased sugar formation begins at once, and the amount of free acid decreases. This occurs especially on the lower side. There is a movement of water from the upper to the lower side. Thus the concentration of sugar in the juice of the lower side becomes less than in that of the upper.

Miss SCHLEY (9), working with shoots of etiolated *Vicia Faba* seedlings, found rather complex changes in the titration acidity after exposure to gravity. First the concave side was more acid, then the convex, then they became about equal while bending was in progress. After the tip had passed the vertical, the concave side became the more acid, but this difference gradually disappeared. She found the water content somewhat greater on the convex side,

but the samples were taken after bending was practically complete. The percentage of sugar in the convex flank was considerably lower than in the concave, after an exposure of 45 minutes.

In various roots exposed to gravity CZAPEK (3) found an accumulation of intermediate products of oxidation of certain amino acids, due to the presence of an antienzyme which inhibits the normal oxidation of these substances. He found no differences between the upper and lower flanks in this respect. GROTTIAN (7) and GRAFE and LINSBAUER (5) were unable to confirm CZAPEK's results. The latter workers (6) found that geotropic response causes no differences in catalase activity.

SMALL (10) found increased permeability in the cortical cells of both sides of root tips of *Vicia Faba* when exposed to gravity. The permeability of the lower sides showed a greater increase than that of the upper side.

Changes in the viscosity of the protoplasm during geotropic stimulation were studied by WEBER (11), who found that the viscosity is lessened. ZOLLIHOFER (12) was unable to confirm this result, and states that the method used is subject to large experimental errors.

Experimental work

The first material used in this work was nodes of corn that had completed their growth. The node was cut out, together with about half the internodes above and below, and the sheath removed. The node was then planted horizontally in a bank of moist sand in a box from which light was excluded. This material is especially good because no growth occurs aside from that due to the action of gravity, and because the region which bends in most cases is very clearly defined. After exposure to gravity this region was cut out and divided into upper and lower flanks. There are at least two objections to the use of corn nodes. First, suitable material can be obtained only during a comparatively short time each year. Second, whether a given node will respond to gravity is very uncertain. Some nodes that apparently were healthy and in good condition did not respond at all, and others which showed no evident differences responded readily. This makes practically impossible a study of the period before visible bending begins.

Etiolated *Vicia Faba* seedlings were used for the later work. For the moisture and titration acidity determinations the plants were grown in moist sphagnum in pans. When the shoots had reached a suitable length (6–8 cm.) they were exposed to gravity by setting the pans on edge. In collecting the material, the leaf was removed and the stem divided as accurately as possible into upper and lower flanks. The terminal 3–4 cm. were used. For the other work the plants were grown in moist sawdust in a dark cool room.

TABLE I

MOISTURE AND ACIDITY IN CORN NODES EXPOSED TO GRAVITY

TIME OF EXPOSURE	MOISTURE			ACIDITY IN CC. 0.05 *N* NaOH PER GM. FRESH WEIGHT		
	Upper flank (percentage)	Lower flank (percentage)	Difference (percentage)	Upper flank	Lower flank	Difference
Hours						
3.....	86.68	87.10	+0.42	0.49	0.48	−0.01
3.....	87.00	86.83	−0.17	0.47	0.47	.....
6.....	86.85	86.98	+0.13	0.47	0.53	+0.06
6.....	87.09	87.18	+0.09	0.51	0.59	+0.08
9.....	84.97	84.43	−0.54	0.46	0.48	+0.02
9.....	84.04	85.10	+1.06	0.51	0.54	+0.03
12.....	83.80	83.19	−0.61	0.49	0.50	+0.01
12.....	83.10	82.72	−0.38	0.57	0.59	+0.02
15.....	85.50	84.61	−0.89	0.47	0.46	−0.01
15.....	84.24	84.26	+0.02	0.47	0.54	+0.07
18.....	83.50	83.71	+0.21	0.38	0.48	+0.10
18.....	82.39	82.79	+0.40	0.51	0.55	+0.04
21.....	82.35	83.40	+1.05	0.61	0.65	+0.04
21.....	82.31	82.71	+0.40	0.67	0.70	+0.03
24.....	83.73	82.99	−0.74	0.55	0.57	+0.02
24.....	82.97	82.90	−0.07	0.58	0.65	+0.07
27.....	81.39	82.19	+0.80	0.64	0.57	−0.07
27.....	81.44	82.44	+1.00	0.65	0.76	+0.11

When they had reached a suitable length they were transferred to boards where they were held in place by pieces of cork. The boards were placed upright in a large galvanized iron container, under a spray. They were kept in this position for at least 24 hours, and then exposed to gravity by rotating the board through 90°.

In the determination of moisture the corn nodes were dried to constant weight in vacuo at 80° C. The samples varied in weight from 2 to 5 gm., according to the number and size of the nodes used. Table I gives the results of the series in which the

nodes were exposed to gravity for varying lengths of time, from 3 to 27 hours. In the last column, + is in favor of the convex side and − in favor of the concave. This method of statement is used in all the tables. As already mentioned, corn nodes are not at all uniform in their response to gravity, and because of this fact a second set was run in which nodes that had bent approximately to the degree indicated were used. The results will be found in table II.

TABLE II

MOISTURE AND ACIDITY IN CORN NODES EXPOSED TO GRAVITY

DEGREE OF BENDING	MOISTURE			ACIDITY IN CC. 0.05 *N* NaOH PER GM. FRESH WEIGHT		
	Upper flank (percentage)	Lower flank (percentage)	Difference (percentage)	Upper flank	Lower flank	Difference
0	82.41	81.30	−1.11	0.62	0.65	+0.03
5	80.27	80.08	−0.19	0.72	0.75	+0.03
5	81.19	80.44	−0.75	0.60	0.73	+0.13
5	84.68	84.21	−0.47	0.60	0.63	+0.03
10	80.42	80.77	+0.35	0.75	0.83	+0.08
10	86.04	86.35	+0.31	0.56	0.60	+0.04
15	86.31	87.63	+1.32	0.65	0.72	+0.07
20	85.13	87.60	+2.47	0.66	0.80	+0.14
25	87.12	89.52	+2.40	0.80	0.76	−0.04
25	87.52	89.29	+1.77	0.66	0.71	+0.05

Individual differences in moisture content are so great that different samples cannot be compared. It is only possible to compare opposite flanks of the same sample. In general the differences are slight, and in view of the high percentage of moisture present they may not be significant. There are some features of the results which are of interest, however, especially when the two sets are compared. In the time of exposure set the differences are variable, but in general favor the convex side up to 9 hours of exposure. At 12 and 15 hours, when bending is well started, there is a decided difference in favor of the concave side. At 18, 21, and 27 hours the convex side contains much more moisture. The results at 24 hours appear to be anomalous, especially as no corresponding change is found in the other set. In the degree of bending set the differences are more regular and more marked. During the early stages of bending the concave flank contains the more moisture, but

as bending proceeds the convex flank contains more water. The same difference is indicated in the time of exposure set, but because of irregularities in the response of the nodes, it is not so obvious.

The results with *Vicia Faba* shoots are given in table III. The fresh samples weighed about 1 gm. They were dried to constant weight at 100–102° C. The differences are so small and so

TABLE III

MOISTURE AND ACIDITY IN *Vicia Faba* SHOOTS EXPOSED TO GRAVITY

TIME OF EXPOSURE	MOISTURE			ACIDITY IN CC. 0.05 *N* NaOH PER GM. FRESH WEIGHT		
	Upper flank (percentage)	Lower flank (percentage)	Difference (percentage)	Upper flank	Lower flank	Difference
15 minutes...	93.35	93.35	.....	1.40	1.15	−0.25
15 minutes...	93.33	93.33	.....	1.18	1.18	.....
30 minutes...	92.43	92.50	+0.07	1.10	1.15	+0.05
30 minutes...	93.25	93.13	−0.12	0.99	1.05	+0.06
45 minutes...	92.48	91.63	−0.85	1.05	1.12	+0.07
45 minutes...	92.67	92.73	+0.06	1.07	0.94	−0.13
1 hour.....	93.02	93.19	+0.17	1.16	1.19	+0.03
1 hour.....	92.40	92.50	+0.10	1.20	1.16	−0.04
2 hours.....	91.53	91.50	−0.03	1.54	1.58	+0.04
2 hours.....	93.00	93.03	+0.03	1.39	1.37	−0.02
3 hours.....	92.50	92.65	+0.15	1.18	1.20	+0.02
3 hours.....	92.13	92.45	+0.32	1.15	1.10	−0.05
5 hours.....	92.50	92.80	+0.30	1.23	1.17	−0.06
5 hours.....	92.95	93.15	+0.20	1.18	1.10	−0.08
7 hours.....	92.70	92.63	−0.07	1.02	0.91	−0.11
7 hours.....	92.60	92.60	.....	1.13	1.08	−0.05
9 hours.....	92.37	92.70	+0.33	1.13	1.14	+0.01
9 hours.....	92.87	92.93	+0.06	1.25	1.22	−0.03
11 hours.....	92.35	92.00	−0.35	1.13	1.11	−0.02
11 hours.....	92.65	92.65	.....	1.13	1.19	+0.06
13 hours.....	92.69	92.80	+0.11	1.15	1.12	−0.03
13 hours.....	92.87	92.73	−0.14	1.15	1.12	−0.03
17 hours.....	92.97	92.89	−0.08	1.13	1.26	+0.13
17 hours.....	92.25	92.27	+0.02	1.32	1.41	+0.09
21 hours.....	91.60	91.60	.....	1.15	1.11	−0.04
21 hours.....	93.00	93.07	+0.07	0.97	0.95	−0.02

irregular as to be insignificant. At the periods from 1 to 9 hours the convex side seems to contain, in general, a little more moisture, but the differences are too slight to serve as a basis for any conclusions.

For the determination of titration acidity the samples were ground in a mortar with sand which had been treated with HCl and washed free from acid. Fifty cc. of water was added and the mixture titrated to phenolphthalein with 0.05 *N* NaOH. Blanks

were run on the sand and water, and were used to correct the results. There was not enough color in the material to interfere seriously with the phenolphthalein endpoint, but the endpoint is somewhat slow, and, especially with material containing so little acid, the unavoidable errors are apt to cause differences which represent a large percentage of the total titration. The results for corn nodes, calculated as cubic centimeters 0.05 *N* NaOH per gram of fresh material, are given in tables I and II. The differences found between the two flanks are small. The convex side seems quite uniformly to be the more acid.

A few measurements of the hydrogen ion concentration of the press juice of corn nodes which had bent from 5° to 15° were obtained. The measurements were made electrometrically, using a modified form of the Barendrecht electrode. The following P_H values were obtained, that for the upper flank being given first in each case: 4.919, 5.012; 5.136, 5.246; 5.104, 5.198. In these three cases, therefore, the hydrogen ion concentration of the juice of the concave flank was the greater, although, as has been noted, the titration acidity varied quite uniformly in the other direction.

The titration results with *Vicia Faba* are given in table III. The differences are slight and irregular, and do not correspond at all closely with those reported by Miss SCHLEY.

Determinations of hydrogen ion concentration, and electrometric titrations, were made on the press juice of the upper and lower flanks of *Vicia Faba* seedlings that had been exposed to gravity. The material was frozen immediately after collection. A special hand press was used which would remove the juice very completely from samples containing not more than 10 gm. of the fresh material. Five cc. of the juice was taken for the determination. The hydrogen ion concentration was determined immediately, after adding 1 cc. of 0.10 *N* NaOH free from carbonates. This is practically the method used by EMSLANDER (4) in his work with beer. Preliminary experiments showed that the part of the titration curve including these two points is always, for this material, the straight line part of the curve which crosses the neutral line. Usually the two points obtained were on opposite sides of neutrality, so that the cubic centimeters of 0.10 *N* NaOH required to titrate to $P_H = 7.0$

could be calculated by interpolation. In only one case was it necessary to extrapolate.

In table IV are given the P_H values of the press juice, and the cubic centimeters of 0.10 *N* NaOH required to bring 5 cc. of the juice to the neutral point. The results obtained on right and left halves of seedlings not exposed to gravity are given in the last two lines of the table. These results show the magnitude of the differences that might arise from other causes than the action of gravity, such as actual differences between two sides of a plant, and errors in measurement. In a few cases the differences found

TABLE IV

ELECTROMETRIC DETERMINATIONS ON PRESS JUICE OF *Vicia Faba* SHOOTS EXPOSED TO GRAVITY

TIME OF EXPOSURE	HYDROGEN ION EXPONENT			ACIDITY IN CC. 0.10 *N* NaOH PER 5 CC. OF JUICE		
	Upper flank	Lower flank	Difference	Upper flank	Lower flank	Difference
30 minutes...	6.124	6.198	+0.074	0.81	0.77	−0.04
30 minutes...	6.122	6.060	−0.062	0.89	1.05	+0.16
1 hour.....	6.127	6.207	+0.080	0.81	0.71	−0.10
1 hour.....	6.137	6.092	−0.045	0.83	0.92	+0.09
2 hours.....	6.144	6.198	+0.054	0.75	0.77	+0.02
2 hours.....	6.132	6.160	+0.028	0.79	0.75	−0.04
4 hours.....	6.203	6.060	−0.143	0.74	0.81	+0.07
4 hours.....	6.170	6.193	+0.023	0.72	0.75	+0.03
Not exposed.	6.079	6.102	+0.023	0.88	0.82	−0.06
Not exposed.	6.048	6.103	+0.055	0.87	0.79	−0.08

between the flanks of plants acted on by gravity are greater than those in the blank determinations, but where this is the case the differences are not regular in direction.

The plan of the work included as complete a study as possible of the various oxidizing enzymes. Only the catalase had been studied when it became necessary to discontinue the work. Determinations of catalase activity were made by the method of APPLEMAN (1), as modified and used by CROCKER and HARRINGTON (2). Catalase activity decreases from the tip downward, and it is not exactly proportional to the weight of the sample. It was not possible entirely to avoid the errors from both of these sources. The following method was used. After exposure to gravity the

shoot was divided as accurately as possible into upper and lower flanks. A sample was cut from one of the flanks, starting at the tip and going as far as was necessary to obtain exactly 0.200 gm. The other flank was left attached to the plant, and kept in a moist dark place while catalase was determined in the first sample. The second flank was then sampled in the same way as the first, and its catalase content determined. Six plants were used for each period of exposure. The catalase content of the upper flank of three of these was determined first, that of the lower flank of the other three first. The 0.200 gm. sample was ground for 2 minutes in a mortar with sand and a little $CaCO_3$. It was then washed into the apparatus with 15 cc. of water. After the apparatus had reached the temperature of the bath, 5 cc. of H_2O_2 (dioxygen), neutralized with a little $CaCO_3$, was added. Shaking was begun at once, and readings of the volume of oxygen evolved were taken every minute for 10 minutes. The bath was kept at 25° C. and the air temperature did not change significantly during any single set of determinations.

The results given in table V are the cubic centimeters of oxygen evolved in 10 minutes. The average of the results for each of the periods of exposure is in favor of the upper flank, but only in the case of the 1 hour samples were all the results in this direction. In the other sets the individual results vary so widely that no conclusions can be drawn from the averages.

For chemical analysis samples of about 100 gm. fresh weight were used. These were collected in flasks containing 0.5 gm. $CaCO_3$ and sufficient alcohol so that the final concentration was approximately 80 per cent. It was during the collection of the last of these samples that it became necessary to drop the work. In order that the material might not be lost, H. A. JONES consented to complete the collection and carry out the analyses. The writer wishes to express his thanks to Dr. JONES for his kindness in making this addition to the data possible.

The soluble and insoluble portions were separated, and total solids determined in each. Sugars were determined as follows. Aliquots of the extract were evaporated to remove alcohol, taken up with water, and clarified with basic lead acetate. The excess lead

was removed by Na_2SO_4. In the filtrate reducing sugars were determined before and after subjecting it to the standard method for the hydrolysis of sucrose by HCl. The Bertrand titration method was used for determining the amounts of copper reduced. The results are expressed as glucose and sucrose respectively, although it is recognized that other sugars are undoubtedly included. Total nitrogen was determined in both the soluble and insoluble

TABLE V

CATALASE ACTIVITY IN SHOOTS OF *Vicia Faba* EXPOSED TO GRAVITY (EXPRESSED AS CUBIC CENTIMETERS OF OXYGEN LIBERATED BY 0.20 GM. OF MATERIAL)

Time of exposure	Upper flank	Lower flank	Difference
30 minutes	7.15	7.80	+0.65
30 minutes	8.20	8.00	−0.20
30 minutes	8.45	7.50	−0.95
30 minutes	8.40	6.30	−2.10
30 minutes	9.00	10.70	+1.70
30 minutes	7.10	6.70	−0.40
1 hour	9.85	8.65	−1.20
1 hour	9.40	8.20	−1.20
1 hour	12.20	11.40	−0.80
1 hour	10.20	9.00	−1.20
1 hour	8.00	7.50	−0.50
1 hour	8.80	8.70	−0.10
2 hours	9.85	10.05	+0.20
2 hours	8.80	9.00	+0.20
2 hours	10.10	9.95	−0.15
2 hours	11.30	11.00	−0.30
2 hours	7.10	7.40	+0.30
2 hours	8.60	7.60	−1.00
4 hours	9.25	9.30	+0.05
4 hours	7.40	7.10	−0.30
4 hours	5.15	6.60	+1.45
4 hours	8.80	8.50	−0.30
4 hours	7.10	6.60	−0.50
4 hours	8.65	7.60	−1.05

portions by the Kjeldahl method. The results are given in table VI. The differences in direct reducing sugars, "glucose," are comparatively slight. Those in reducing sugars formed on hydrolysis, "sucrose," are considerably greater, especially when figured as percentages of the total. It is to be remembered, however, that the total amount of sucrose is relatively small, and that the errors in both determinations may accumulate in that of sucrose. It seems

to be impossible to correlate the differences found with the process of bending. The same may be said of the distribution of nitrogen.

Summary

Definite moisture changes accompany geotropic bending in corn nodes. During the early stages of bending there is a greater percentage of moisture in the concave flank. When the process has developed the percentage of water is greater in the convex flank.

Although titration acidity is greater in the convex flank, the differences are very slight. The results on hydrogen ion concentration, although uniform in direction, are not numerous enough to serve as a basis for conclusions.

It is impossible, with the data obtained, to correlate the geotropic bending of etiolated *Vicia Faba* shoots with differences in

TABLE VI

ANALYSES OF *Vicia Faba* SHOOTS EXPOSED TO GRAVITY (IN PERCENTAGE OF FRESH WEIGHT)

Time of Exposure	Upper flank	Lower flank	Difference	Upper flank	Lower flank	Difference
	Glucose			Sucrose		
30 minutes...	2.16	2.15	−0.01	0.450	0.279	−0.171
1 hour.....	1.47	1.40	−0.07	0.187	0.287	+0.100
2 hours.....	1.56	1.67	+0.11	0.221	0.269	+0.048
4 hours.....	1.37	1.41	+0.04	0.449	0.289	−0.160
	Total sugars			Moisture		
30 minutes...	2.61	2.43	−0.18	91.57	92.39	+0.82
1 hour.....	1.65	1.69	+0.04	92.54	92.46	−0.08
2 hours.....	1.78	1.94	+0.16	91.62	91.82	+0.20
4 hours.....	1.82	1.70	−0.12	91.93	92.13	+0.20
	Soluble nitrogen			Insoluble nitrogen		
30 minutes...	0.294	0.301	+0.007	0.302	0.284	−0.018
1 hour.....	0.261	0.259	−0.002	0.303	0.306	+0.003
2 hours.....	0.279	0.305	+0.026	0.314	0.296	−0.018
4 hours.....	0.264	0.258	−0.006	0.349	0.324	−0.025

moisture, titration acidity, hydrogen ion concentration, catalase activity, or the distribution of sugars and nitrogen containing substances.

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